REMARKS

I. Status Summary

Claims 1-17 are pending in the present U.S. patent application and have been examined.

Claims 1-17 have been rejected under 35 U.S.C. § 112, first paragraph, upon the contention that the specification does not reasonably provide enablement for a method of screening for susceptibility to sub-optimal norepinephrine (NE) transport in a subject.

Applicants acknowledge that the rejections under 35 U.S.C. § 103(a) presented in the previous Official Action have been withdrawn. Applicants would like to thank Examiner Chunduru for withdrawing the two obviousness rejections in view of applicants' amendments and remarks presented in response to the previous Official Action.

Reconsideration of the application based on the remarks sets forth herein below is respectfully requested.

II. Response to the Claim Rejections under 35 U.S.C. § 112, First Paragraph

Claims 1-17 remain rejected under 35 U.S.C. § 112, first paragraph, upon the contention that the specification, while being enabled for a method of screening for susceptibility to sub-optimal norepinephrine (NE) transport in orthostatic intolerance (OI), does not reasonably provide enablement for a method of screening for susceptibility to sub-optimal NE transport in a subject. According to the United States Patent and Trademark Office (hereinafter "the Patent Office"), the specification does not enable any person skilled in the art to which it pertains to use the invention commensurate in scope with the claims.

After careful consideration of the rejection and the Patent Office's bases therefor, applicants respectfully traverse the rejection and submit the following remarks.

In support of its rejection, the Patent Office asserts that the specification does not demonstrate any correlation between NE transport in general with any specific

mutation except for the correlation between the A457P mutation in orthostatic intolerance, thus the specific mutation was correlated with NE transport in orthostatic intolerance and not with general NE transport in a subject.

With regard to this assertion, applicants respectfully submit that the Patent Office offers no sound scientific basis for asserting that a mutation that results in an amino acid change in the norepinephrine (NE) transporter (NET) gene product that results in sub-optimal NE transport in one group of patients would not be expected to result in sub-optimal NE transport in another group of patients. Applicants respectfully submit that the gene in question is the NE transporter itself, and a mutation in NET would be predicted to function in OI patients and non-OI patients similarly with respect to NE transport. Thus, applicants respectfully submit that the Patent Office's implicit assertion that transport through an NET polypeptide having an A457P in OI patients would be expected to be different than transport through an NET polypeptide having an A457P in non-OI patients cannot be supported by adequate scientific or technical reasoning as is required under M.P.E.P. Section 2164.04. Thus, this assertion fails to support the instant rejection under 35 U.S.C. § 112, first paragraph.

Further, the Patent Office concedes that the specification enables a method for screening for susceptibility to sub-optimal NE transport in subjects with OI. The method itself recites (a) obtaining a biological sample from the subject; and (b) detecting a polymorphism of a NE transporter gene encoding an amino acid change in the biological sample from the subject. Neither of these two steps is dependent on any characteristic that is unique to patients with OI, and thus the method does not depend on the subject having OI in order to be practiced by the skilled artisan.

Thus, applicants respectfully submit that the specification as filed broadly enables a method for screening for sub-optimal NE transport in a subject, and further that the disclosure that enables the method in subjects with OI is equally applicable to subjects otherwise suspected of suffering from sub-optimal NE transport. The method is not limited to subjects with OI, as there is no characteristic or condition associated with OI that uniquely limits the method to only those patients. If, as the Patent Office concedes, the method is enabled for OI patients (who, applicants wish to point out, are

exemplary "subjects"), applicants respectfully submit that the method can be applied to other subjects without modification.

Consequently, applicants respectfully submit that the Patent Office has created an artificial distinction between subjects that are OI patients and subjects generally, which distinction that is not supported by any scientific reasoning. Applicants thus respectfully submit that the Patent Office has not satisfied the requirements of MPEP § 2144.03 and In re Zurko (258 F.3d 1379,1385), which state that "an assessment of basic knowledge and common sense that is not based on any evidence in the record lacks substantial evidence support".

Furthermore, applicants respectfully submit that 35 U.S.C. §112, first paragraph, requires no more than a disclosure sufficient to enable one skilled in the art to carry out the invention commensurate with the scope of the claims, and this requirement has clearly been met. The identification of the A457P polymorphism and the associated amino acid change in the NET polypeptide with sub-optimal NE transport in OI patients provides for the first time a connection between a polymorphism in the NET gene and a sub-optimal NE transport phenotype. Applicants respectfully submit that this provides a solid basis for analysis of NET polymorphisms generally and as presently claimed.

Additionally, the specification discloses SEQ ID NOs: 1 and 2, which are the nucleotide and amino acid sequences, respectively, of the human NET gene and gene product. The "MATERIALS AND METHODS USED IN EXAMPLES" section of the specification also discloses techniques that can be used to isolate and characterize NET nucleic acids from a subject in order to identify polymorphisms in any exon of the NET coding sequence (see the subsection entitled Detection of Mutations). Once polymorphisms have been identified, the specification of the instant application teaches assays that can be used for determining whether or not a polypeptide encoded by the polymorphic NET nucleic acids would be expected to result in sub-optimal NE transport (*see e.g.*, the section of the Examples entitled Functional Analysis of Identified Coding Mutation). Applicants respectfully submit that the Chinese hamster ovary transfection assay used to test the A457P polymorphism can

be adapted for testing any nucleic acid sequence encoding a polymorphic NET polypeptide by one of ordinary skill in the art upon a review of the present disclosure. The specification also discloses various pharmacological tests that can be performed on subjects that can be used as further evidence of in vivo sub-optimal NET function (*see e.g.*, the section entitled Other Diagnostic Methods beginning on page 81).

In response to this showing, the Patent Office offers no more than conclusory statements that do not address the rebuttal presented by applicants. For example, the Patent Office contends that applicants' arguments were not persuasive because "the specification enables a method of screening a subject with OI for sub-optimal NE transports, wherein the method clearly associates a specific mutation (A457P) with a subject with OI and does not provide any polymorphism in NE transport in general [that] is associated with sub-optimal NE transport". Official Action at page 7. Applicants respectfully submit that the A457P mutation is a mutation in the norepinephrine transporter (NET), and is associated with sub-optimal NE transport. The presence of this mutation in subjects with OI does not exclude the possibility that patients that do not have OI might also have the mutation and suffer from sub-optimal NE transport.

The Patent Office further asserts that the specification does not provide enablement that the specific mutation (A457P) is associated with sub-optimal NE transport in a subject without OI. Official Action at page 7. The Patent Office offers no scientific basis, however, as to why one of ordinary skill in the art would assume that NE transport in OI patients that have the A457P mutation would be different than NE transport is non-OI subjects that have the A457P mutation. On the contrary, applicants respectfully submit that one of ordinary skill in the art would recognize that sub-optimal transport of norepinephrine would correlate with a mutation in the norepinephrine transporter such as the A457P mutation. Whether or not this mutation always causes OI, as the Patent Office appears to believe, is not strictly required in determining sub-optimal NE transport. As disclosed in the specification, an NE transporter having the A457P mutation is a sub-optimal NE transporter. *See* Figure 2D. Thus, applicants respectfully submit that one of ordinary skill in the art would

recognize that any subject carrying the A457P mutation would be expected to suffer from sub-optimal NE transport regardless of whether or not they had OI.

It is therefore respectfully submitted that the Patent Office cannot establish a *prima facie* case of lack of enablement simply by stating that applicants have not shown sub-optimal NE transport in non-OI patients. No scientific basis has been provided for the implicit assumption upon which this assertion must be based that somehow a transporter that shows essentially no transport activity *in vitro* and is associated with sub-optimal NE transport in OI patients *in vivo* would nonetheless function normally in non-OI patients. Thus, applicants respectfully submit that this assertion does not support the Patent Office's *prima facie* case of lack of enablement.

Next, the Patent Office asserts that applicants' arguments regarding the ability to assay NE transport activity using the CHO cell transfection assay are not persuasive because "only some mutations in the NET gene would result in altering an amino acid and result in an abnormal peptide. The specification does not enable that all polymorphisms of the NET gene would result in an aberrant polypeptide and result in sub-optimal NE transport". Official Action at page 7. Applicants respectfully submit that even assuming *arguendo* that only some mutations would result in abnormal peptides, this does not address the usefulness of the CHO cell transfection assay or negate that the CHO assay supports the enablement of the claims.

Initially, applicants respectfully submit that one of ordinary skill in the art would be able to eliminate a vast number of mutations from the screening method because such mutations do not alter the polypeptide sequence. Exemplary mutations would include any mutation that does not encode an amino acid change. However, claim 1 recites detecting a polymorphism of a NE transporter gene encoding an amino acid change, so these mutations are eliminated from the screening method by virtue of the express language of the claim.

Furthermore, the identification of the A457P polymorphism with sub-optimal NE transport in OI patients provides for the first time a connection between a polymorphism in the NET gene and a sub-optimal NE transport phenotype. Applicants respectfully submit that this provides a solid basis for analysis of NET polymorphisms

generally and as presently claimed. Thus, applicants respectfully submit that any mutation that results in an amino acid change would be a candidate for screening for sub-optimal NE transport. Once these mutations are identified in a subject, the CHO assay disclosed in the specification can be used to confirm that the encoded NET is characterized by sub-optimal activity. In rebuttal, the Patent Office offers no scientific basis that adequately addresses applicants' contention that mutations in the NET gene can result in sub-optimal NE transport, and that when such mutations are identified in a subject, the NE transport activity of the mutant NET polypeptide can be assayed using the CHO assay. Rather, the Patent Office asserts that "the techniques disclosed in the specification enable a screening method for susceptibility top sub-optimal NE transport in subjects with OI but not for subjects without OI". Official Action at page 8. As discussed in more detail hereinabove, however, this latter assertion is not supported by suitable scientific reasoning, and thus fails to support the Patent Office's contentions regarding applicants' arguments, particularly when considered in light of the activity of the A457P mutant polypeptide in the CHO assay, which removes the NET polypeptide from the environment of the OI patient.

Furthermore, experiments related to NET mutations and transporter activity have continued in the laboratories of the co-inventors. Subsequent work has identified several additional mutations that affect NET activity. This data is presented in true and accurate form in **Exhibit A**, attached hereto, which demonstrates that other NET mutations, including R121Q, N292T, A369P, F528C, and Y548H, all of which were identified in natural populations, result in altered NE transport. Accordingly, applicants respectfully submit that the identification of the A457P polymorphism with sub-optimal NE transport in OI patients demonstrates that there is an association between a polymorphism in the NET gene encoding an amino acid change and a sub-optimal NE transport phenotype. Applicants respectfully submit that this provides a solid basis for analysis of NET polymorphisms generally and as presently claimed

Summarily, applicants respectfully submit that the specification as filed teaches techniques that can be used for identifying nucleic acids encoding polymorphic NET polypeptides present in subjects, and further for assaying the NE transport activities of

these polypeptides to identify polymorphisms that cause sub-optimal NE transport. Accordingly, applicants respectfully submit that the current rejection of claims 1-17 under 35 U.S.C. § 112, first paragraph, has been addressed. Applicants respectfully request the withdrawal of the instant rejection, and the allowance of claims 1-17.

III. Discussion of the New Claim

New claim 80 has been added. Support for the new claim can be found throughout the specification as filed, including particularly in the claims as originally filed (for example, original claims 1, 2, and 5). Additional support can be found on page 11, lines 3-16; on page 12, lines 4-8; and in Example 4. Thus, no new matter has been added with the inclusion of new claim 80.

Applicants respectfully submit that the remarks presented hereinabove with respect to the rejection under 35 U.S.C. § 112, first paragraph, are equally applicable to new claim 80. Accordingly, new claim 80 is believed to be in condition for allowance, and applicants respectfully solicit a Notice of Allowance to that effect.

CONCLUSIONS

In light of the above Remarks it is respectfully submitted that the present application is now in proper condition for allowance, and such action is earnestly solicited.

If any minor issues should remain outstanding after the Examiner has had an opportunity to study the Amendment and Remarks, it is respectfully requested that the Examiner telephone the undersigned attorney so that all such matters may be resolved and the application placed in condition for allowance without the necessity for another Action and/or Amendment.

DEPOSIT ACCOUNT

The Commissioner is hereby authorized to charge any deficiencies or credit any overpayments associated with the filing of this correspondence to Deposit Account Number **50-0426**.

Respectfully submitted,
JENKINS, WILSON & TAYLOR, P.A.

Date: March 22, 2005 By: Arles A. Taylor, Jr.

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Exhibit A

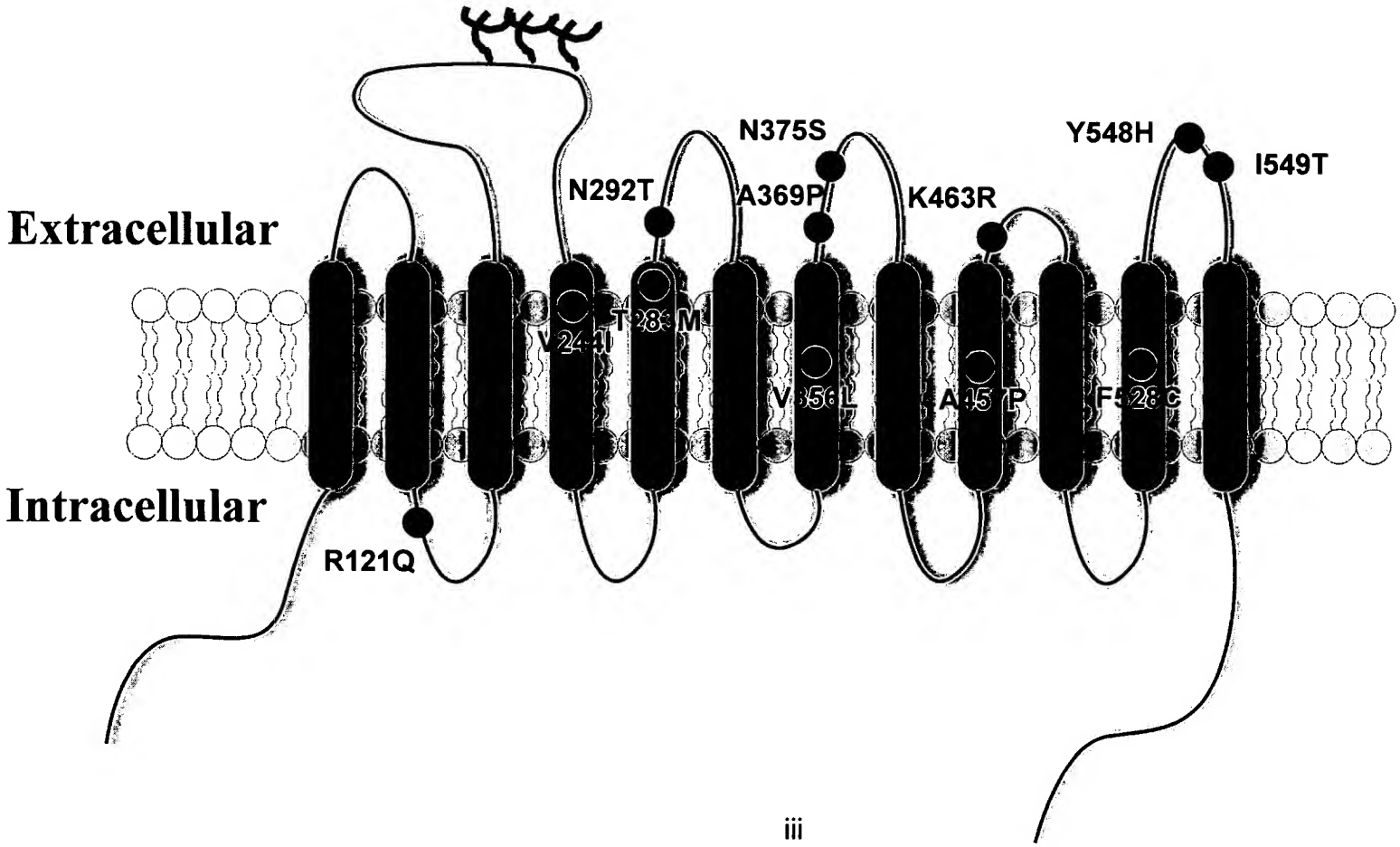
1. Positions of Reported hNET Variants: locations of coding variants of hNET in screens of human subjects with cardiovascular, ADHD, or non-specific diagnoses
2. hNET Variant Substrate Transport: functional Impact of hNET coding variants in transfected COS-7 cells. Each variant was transfected in parallel with wild type hNET and evaluated for transport activity using [^{3}H]NE or [^{3}H]DA as substrate. Asterisks indicate significant differences from control activity, $p < 0.05$, ANOVA followed by Fisher's exact test.
3. hNET Variant Effects on Protein Expression in Total Cell Extracts: protein expression impact of hNET coding variants in transfected COS-7 cells. Each variant was transfected in parallel with wild type hNET and evaluated by western blotting for protein expression using NET specific antisera. Marks noted on the left side of the figure are the migration positions for (bottom to top, non-glycosylated, core N-glycosylated, mature glycosylated and multimeric NET proteins, respectively). Size markers for bands are noted on the right in kDa.
4. hNET Variant Effects on Protein Expression in Total Cell Extracts: protein expression impact of hNET coding variants in transfected COS-7 cells. Each variant was transfected in parallel with wild type hNET and evaluated by western blotting for protein expression using NET specific antisera. Quantitation of blots as developed in Item 3. Here presented are relative levels of the immature 54 kDa and mature 80 kDa species in total extracts. * $p < 0.05$ significantly different from control hNET, ANOVA followed by Fisher's exact test.
5. hNET Variant Effects on Cell Surface Expression: cell surface expression impact of hNET coding variants in transfected COS-7 cells. Each variant was transfected in parallel with wild type hNET and evaluated by western blotting for protein expression using NET specific antisera after biotinylation with a membrane impermeant biotinylating reagent to recover

the surface-specific fraction. Marks noted on the left side of the figure are the migration positions for (bottom to top, non-glycosylated, core N-glycosylated, and mature glycosylated NET proteins respectively). Size markers for bands are noted on the right in kDa.

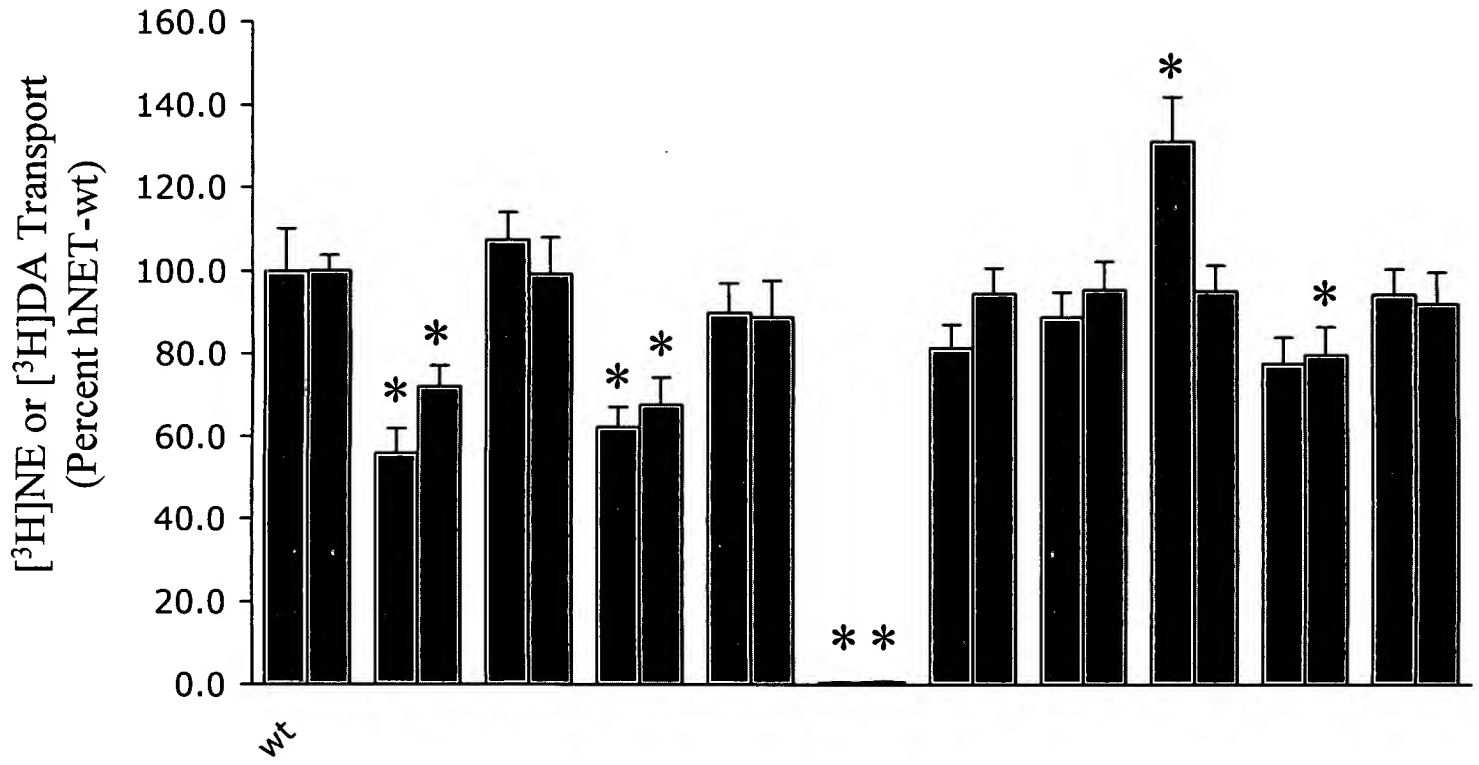
6. hNET Variant Effects on [^{3}H]NE Transport: altered transport kinetics of hNET coding variants. Transporters showing altered NE transport activity in single concentration assays were subjected to increasing concentrations of NE to determine substrate K_m and transport capacity (K_m). Values are tabulated in Item 7.

7. hNET Variant Effects on [^{3}H]NE and [^{3}H]DA Transport: table of kinetic values for coding variant transport activities. hNET coding variants were evaluated for saturable transport activities as in Item 6 and K_m and V_{max} estimates relative to control hNET determined by Least-Squares nonlinear curve fitting (Prism). * $p < 0.05$ significant difference with control hNET

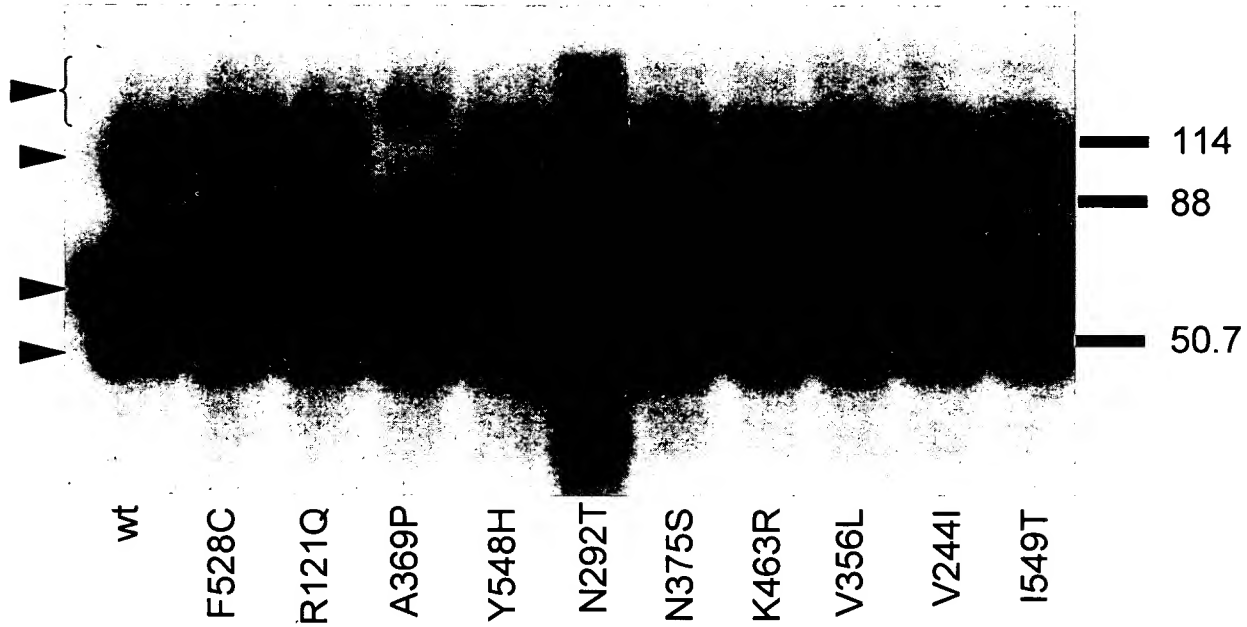
Positions of Reported hNET Variants



hNET Variant Substrate Transport



hNET Variant Effects on Protein Expression in Total Cell Extracts



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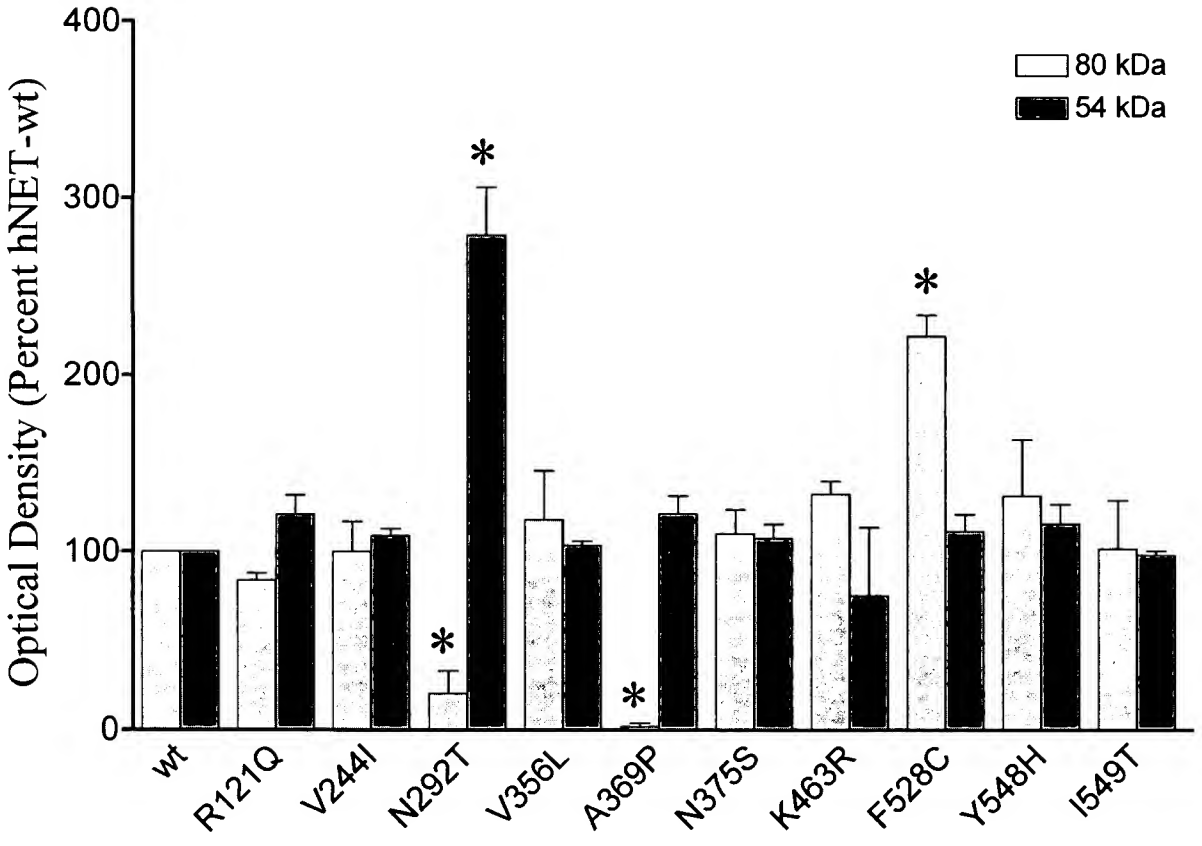
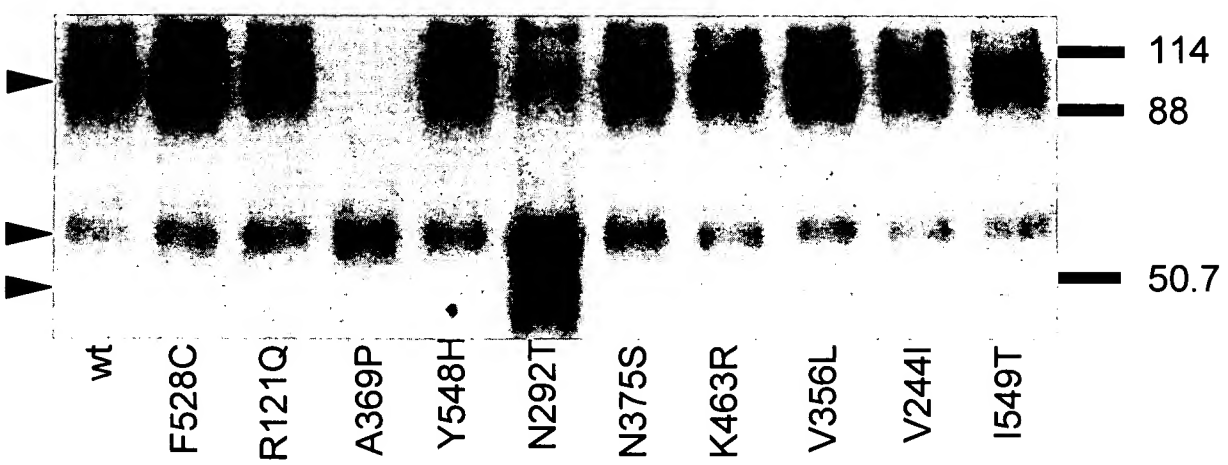
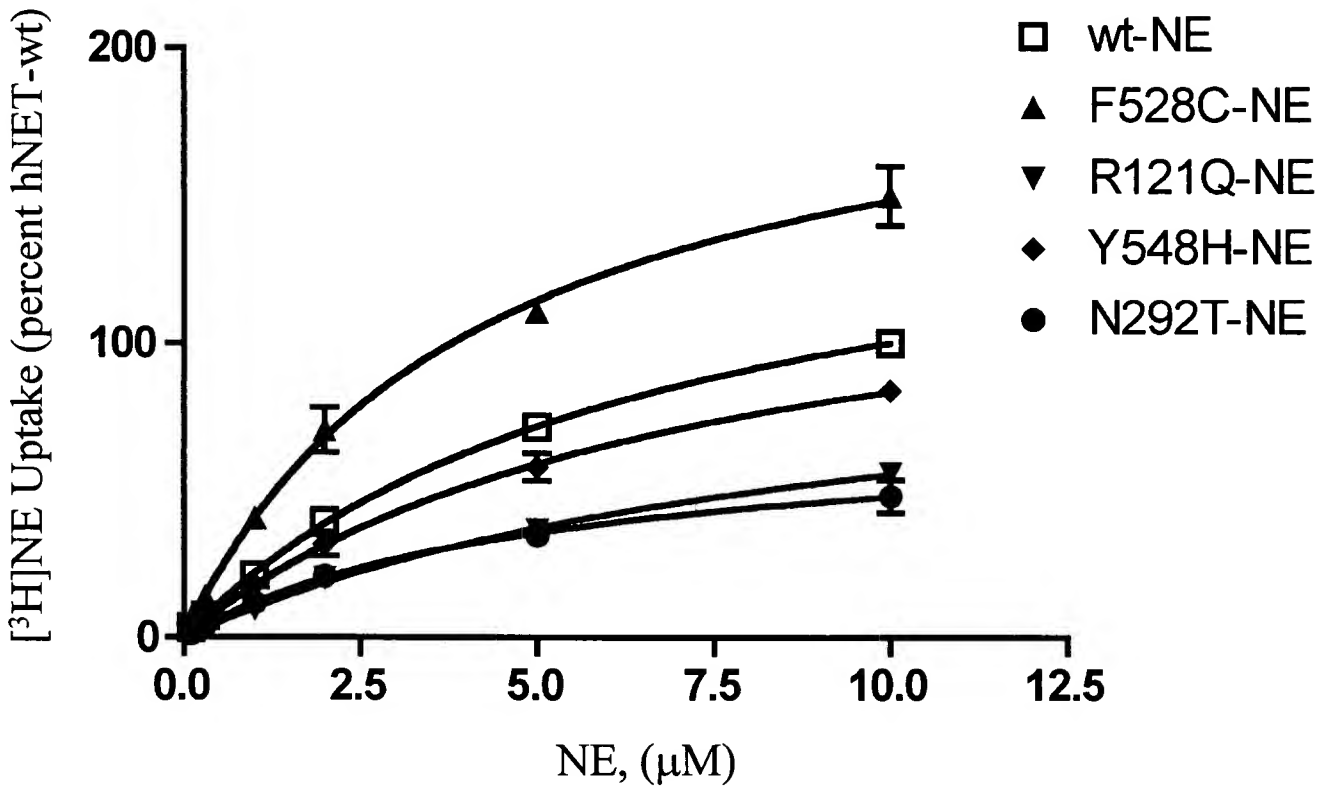
hNET Variant Effects on Protein Expression in Total Cell Extracts



hNET Variant Effects on Cell Surface Expression



hNET Variant Effects on [^{3}H]NE Transport



hNET Variant Effects on [^{3}H]NE and [^{3}H]DA Transport

Table 5: hNET Variant [^{3}H]NE and [^{3}H]DA Saturation Kinetics in COS-7 Cells

hNET Variant	[^{3}H]NE Transport			[^{3}H]DA Transport		
	K_m (μM)	V_{max} (% wt)	V_{max}/ K_m	K_m (μM)	V_{max} (% wt)	V_{max}/ K_m
wt	6.8 ± 1.3	100.0 ± 0.0	3.10 ± 0.21	1.5 ± 0.7	100.0 ± 0.0	6.88 ± 0.96
R121Q	9.8 ± 2.4	65.6 ± 1.4*	1.39 ± 0.25*	1.9 ± 0.9	91.0 ± 8.1	5.05 ± 0.54
N292T	5.2 ± 0.7	43.5 ± 6.3*	1.69 ± 0.17*	1.1 ± 0.5	48.1 ± 5.6*	4.38 ± 0.29*
F528C	4.2 ± 0.7	126.2 ± 14.2*	6.14 ± 0.25*	1.4 ± 0.5	122.6 ± 4.8*	8.74 ± 1.00
Y548H	7.4 ± 0.8	87.8 ± 5.4	2.43 ± 0.22*	1.4 ± 0.6	82.2 ± 3.2*	6.49 ± 0.73

Data are the mean ± S.E.M. of 3 experiments. V_{max} values are expressed as percent of wt transport of NE or DA. Data were analyzed using one-way analysis of variance followed by Fisher's L.S.D., *$p < 0.05$.